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Microbiological Study of Water-Softener Resins

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Microbial identification using effluents backflushed from exhausted urban and rural tank resins and cleaned resins containing the sulfonated copolymer of styrene and divinylbenzene (SDB) were completed, along with microbial assessment of the concentrated stock salt brine. Forty-four different bacterial and fungal genera were identified. Extensive biochemical and animal virulence tests completed on one of the six bacterial salt brine isolates indicated a pathogenic staphylococcal strain. The retention of *Staphylococcus aureus*, a *Flavobacterium sp*, and *Escherichia coli* B bacteriophage was demonstrated both by using the nonexhausted sodium-regenerated resin and by using the same resin exchanged with different mono-, di-, and trivalent cations. Effluent counts completed after bacterial seepage through the resins indicated the Pb^{++}-exchanged resin removed 55% of the bacteria; Na^{+}, Fe^{++}, and Al^{+++} removed 31 to 36% and Ca^{++} and Cu^{++} removed about 10 to 15%. Seventy per cent or more of the bacteriophage was removed by Fe^{++}, Cu^{++}, and Al^{+++}, whereas the Ca^{++} and Na^{+} cations removed 25 to 31%. Over a 77-day period, nonsterile tap water was passed through bacterial seeded and uninoculated SDB (Na) resin columns. Effluent and resin elution counts demonstrated the growth and survival of 2 different bacteria per column. Increased bacterial retention, survival, and multiplication occurred concomitantly with accumulation of organic and inorganic materials and the Ca^{++} and Mg^{++} cations from the tap water. Furthermore, microbial elution from resin particles taken from column depths of 1, 8, and 16 cm indicated a bacterial diminution with increasing depths.

Innumerable studies have described microbial survival and dissemination in public water supply systems. We have contributed to this body of knowledge by relating paralytic poliomyelitis cases to the water-borne virus (1), and by relating septicemia and deaths in prematures to a water-borne *Achromobacter* bacterium (13). Moreover, certain microorganisms indigenous to water, such as the *Flavobacterium* (6), *Pseudomonas* (26), *Alcaligenes* (10), and *Paracolobactrum* (23), have been associated with troublesome nursery infections caused by inhalation therapy equipment in hospitals or contaminated water from other sources (21). Although such infections have not been attributed to or associated with microorganisms cultured from cationic exchange resins used in water-softener units, they are directly involved in water microbiology. It seemed reasonable, therefore, to study these resins. Consider, for example, (i) their microbiological entrapment capabilities, (ii) their favorable environment for microbial maintenance and multiplication, (iii) their longevity (7 to 9 years), and (iv) their reuse capability. Moreover, their microbial dissemination potential cannot be completely discounted when we consider their ubiquitous distribution in water-softener units.

This report describes field and laboratory studies completed on a synthetic cationic exchange resin. Our objectives were simply to provide: (i) a reasonable microbiological assessment of water-softener resins, a study which entailed the generic identification of types and total numbers of the bacteria and fungi recoverable from the first backflush contents of the exhausted water-softener resin tanks servicing mainly urban and rural homes, but also hospitals and dairies; (ii) a microbiological assessment of the 26% "stock salt brine" used to regenerate the cleaned resins; (iii) information regarding bacterial survival time and filtration capabilities using both the nonexhausted sodium-regenerated resin (SDB) and the spent resin, determining whether these phenomena are the same when the resin is exchanged with different mono-, di-, and trivalent cations at the same *p*H, temperature, and flow rate; (iv) information relative to the influence of a progressively changing resin (resin in the process of exhaustion) upon bac-

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terial filtering capacity and survival time; (*v*) an evaluation of affluent and effluent *p*H, chlorine, hardness, and protein levels, finally, relating these factors to the maintenance and multiplication (or both) of the microorganisms in the resin, or conversely, to their inability to survive.

MATERIALS AND METHODS

Sample collection of field studies. Tank sources included predominately urban and rural residences. A limited number were obtained from hospitals and dairies. The 9-inch diameter tanks contained 43 lb. of the SDB resin. Sterile plastic caps, placed on all tank pipe openings when the tanks were collected for cleaning, were removed. Sterile hose connectors were inserted onto the backflush outlet and water inlet pipes. Two gallons of the first backflush of each exhausted tank was collected in sterile 3-gal plastic jars. Similar samples (one per month for 12 months) were obtained from the concentrated stock salt brine. Effluents from 12 separate tanks containing the backflushed, sanitized, regenerated, and washed resin were also cultured.

Enumeration and identification of microorganisms. All samples were processed the same day as collected, or were stored at 4 C and tested the following day. Samples (100-ml) of each shaken, undiluted, backflush sample were filtered using a 47-mm membrane filter (0.45 μm, Millipore Corp., Bedford, Mass.). The contaminated filter pads were placed on plates of Trypticase Soy Agar (TSA; BBL), TSA plates containing 5% defibrinated human blood (BA), Eosin Methylene Blue Agar (EMB; Difco), Salmonella Shigella Agar (SS; Difco), and were incubated at 37 C for 48 hr. Membrane filters were also placed on two plates of Sabouraud Dextrose Agar (SDA, Difco) and Mycophil Agar (MA, BBL). For 2 weeks, one set was incubated at 25 C and the other at 37 C. In addition, one Standard Plate Count Agar (Difco) plate was incubated for 5 days at 55 C and another was incubated for 1 week at 4 C. Anaerobic microorganisms were detected on Anaerobic Agar plates (BBL) incubated in Brewer anaerobic jars at 37 C for 1 week.

Counts were recorded as colony-forming units (CFU) per 100 ml. The TSA plates were used for all counts. If colonies were too numerous to count (more than 300), the backflush samples were diluted 1:10 and 1:100, and the above procedures were repeated.

Generic identification of the different bacterial isolates was based upon colonial characteristics, staining procedures for identification of external and internal structures (including the Gram and acid-fast stain), cellular morphology, and cultural and biochemical characteristics as outlined in the *Manual of Microbiological Methods* (19).

Fungi were identified by their colonial characteristics on SDA, MA, BA, and by their morphological and spore characteristics in lactophenol cotton blue wet mounts and Shoemaker slides. Microbial identification was based on guide lines outlined by Breed and Coake (5, 9).

Stock salt brine isolates. Salt brine dilutions of 1:10 were similarly filtered by membrane filter (Millipore Corp.) and cultured. In addition, 1:100 dilutions were filtered to isolate staphylococcal species on BA. These isolates were cultured in Brain Heart Infusion broth (BHI, Difco). The sources of inoculum for all tests were 24-hr, 37 C cultures. Extensive differential tests were completed for the *Staphylococcus*. Colony characteristics were described after growth for 48 hr at 37 C on Nutrient and Mannitol Salt (MS) Agar plates (Difco). Lactose, glucose, mannitol, xylose, sucrose, and maltose broth fermentation tests (nutrient broth, 0.5% carbohydrate, and phenol red indicator) were completed. Three to four drops of 3% H_2O_2 were added to the growth on Nutrient Agar plates to detect catalase production. Additional tests included nitrate reduction (Trypticase Nitrate Broth, BBL), gelatinase activity (Nutrient Gelatin, Difco), indole production (Tryptone Broth, BBL), Litmus Milk (Difco), and MR-VP medium (BBL). Dissolution of 5 ml of human plasma clotted with thrombin was used to demonstrate fibrinolysin. Deoxyribonuclease Test Agar (Difco) was used to detect deoxyribonuclease activity. An inoculum of 0.05 ml was added to four separate 6-mm agar wells. Phosphatase activity was also determined (3). The slide (7), tube (12), and the Coagulase Agar Base (Difco) methods were used to detect coagulase production. Phage typing was completed by loop spotting phage (National Communicable Disease Center, Atlanta, Ga.) diluted 1:10 on both TSA plates layered with the staphylococci and on soft agar overlay containing the isolates (4). The 24 phages used were: 3A, 3B, 3C, 6, 7US, 29, 42D, 42E, 47, 47C, 52, 52A, 53, 54, 55, 70, 71, 75, 77, 79, 80, 81, 83A, and 187.

Defibrinated (5%) rabbit, sheep and human blood agar plates (Tryptose Blood Agar Base, Difco) were streaked to determine the type of hemolysis. For the hemolysin tube titrations (16), the *Staphylococcus* was grown in 100 ml of Tryptose Phosphate Broth (Difco) in an atmosphere of 20% CO_2 at 37 C for 48 hr. Unfiltered, centrifuged supernatant fluids were diluted for hemolysin determinations against human, rabbit, guinea pig, sheep, and ox red blood cells (RBC). A 2% preparation of RBC (washed three times with saline) was used, and an equivalent amount (0.6 ml) added to 0.85% saline dilutions of the supernatant fluid. Titers were recorded after 1 hr incubation in a 37 C water bath and after overnight (4 C) refrigeration. The titer was the greatest dilution of supernatant fluid that produced 50% hemolysis. Disc antibiotic sensitivity tests (Sensidisc, BBL) were completed on streaked TSA. The following were used: Streptomycin S/10, Chloromycetin C/5, Tetracycline Te/30, Aureomycin A/5, Neomycin N/30, Penicillin P/10, Terramycin T/30, Erythromycin E/15, Triple Sulfa SSS/1.0, Sulfamethoxypyridazine Ky/1.0, Nitrofurantoin Fd/100, Colistin Cl/10, Novobiocin NB/30, Sulfamethizole Th/1.0. The sensitivity tests were positive when a zone of inhibition, regardless of size, appeared around the disc after 24 hr incubation at 37 C.

Animal virulence studies. All tests were completed with an 18-hr staphylococcal BHI culture grown at 37 C. For dermonecrosis studies, two rabbits were injected intradermally in four separate areas with

0.5 ml of the culture (3.2 × 10⁷ cells per ml). Three rabbits were also intravenously inoculated with 0.5 ml of the culture (2.1 × 10⁸ cells per ml). The same cell concentration was used for 0.20-ml intraperitoneal injections in adult Swiss mice (10 mice isolate). If death occurred, internal organs were examined for gross abnormalities and blood agar plates were streaked with peritoneal fluid and heart blood.

Resin characteristics. A synthetic cation-exchange resin (Rohm and Haas Co., Philadelphia, Pa.), consisting of the sulfonated copolymer of styrene and 8% divinylbenzene (SDB) in bead form, was used in all laboratory experiments. The resin has a water content of 44 to 48%. It was produced commercially by treating a polystyrene resin cross-linked by divinylbenzene with fuming sulfuric acid. The sulfonic acid groups are the actual cation exchange functions. Particle size averaged approximately 0.5 mm. The resin can tolerate high temperatures and can be autoclaved at 120°C at 15 lb. of pressure for 15 to 20 min, without affecting the water-softening capabilities. The resin is functional over a *p*H range of 0 to 14. All positive ions are exchangeable, with trivalent ions held more tightly than divalent or monovalent ions. The resin has a service life of 7 to 9 years.

Resin preparation and regeneration. The sodium-regenerated resin (SDB) was shaken and washed three times with deionized water. All ion measurements were completed using water analysis kits supplied by Hach Chemical Co., Ames, Iowa. The following compounds, $CaCl_2 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, $FeSO_4$, $Pb(C_2H_3O_2)_2 \cdot 3H_2O$, $Al_2(SO_4)_3 \cdot 18H_2O$, and $AgNO_3$, were used to replace the sodium ion. A 500-g amount of resin was added to a large column with sterile deionized water. A 0.5 M solution of the cationic solution was passed through each column. The initial, high concentrations of more than 100 ppm of the sodium ion and subsequent absence of the replacement cations in the column effluent indicated a successful exchange. The resin was then washed twice with deionized water, air dried overnight, and stored dry until used.

Resin columns used in microbial filtration and survival studies. The columns consisted of glass tubes with conically shaped bottoms. A perforated glass plate inside the bottom part of the tube supported the resin bed. The column was 30 cm in length and 1.8 cm in width. To efficiently dispense the effluent, rubber tubing was attached to the drawn end of the column to which a glass bell was attached. The flow rate of water (ml/min) was adjusted by means of a screw clamp. A rubber-stoppered glass funnel (3 cm in width and 16 cm in length) was inserted in the upper end of the column. The assembled columns were sterilized in the autoclave.

Resin (20 g) was added to 100 ml of deionized water and autoclaved for 15 min. The cooled slurry was aseptically gravity fed to the sterile column. The length of the resin bed was approximately 18 cm. This was kept covered with 2.5 cm of water at all times to prevent the formation of air bubbles. After the resin had settled for several hours, 100 ml of sterile deionized water was passed through, and 1-ml samples of the effluent were tested for sterility. Triplicate TSA pour plates were prepared for each column.

Cation effect on bacterial filtration. Using the procedures described above, duplicate columns were prepared with resins regenerated with Fe^{++}, Cu^{++}, Ca^{++}, Al^{+++}, Pb^{++}, and Ag^{+}. A 24-hour BHI broth culture (37°C) of *Staphylococcus aureus* was washed three times with sterile deionized water and suspended to give 10^8 cells/ml. During a 15-min period, a 100-ml suspension was passed through each resin column, then 15 ml of sterile deionized water was passed through the column to replace the interstitial water in each resin bed. Serial 10-fold dilutions of the effluents were prepared and triplicate TSA plate counts of each dilution completed. All plates were incubated at 37°C for 24 hr. Sterile, deionized water (100 ml) was again passed through each column at intervals of 3, 7, and 13 days, and plates were prepared and counted as above. The same procedure was repeated with a *Flavobacterium* species isolated from the tap water. All column filtration procedures were carried out at room temperature.

Cation effect on bacteriophage filtration. Resin columns were prepared as described above. *Escherichia coli* B bacteriophage enrichment filtrate was washed three times in deionized water, suspended, and serially diluted. The number of plaque-forming units (PFU) was then determined using the overlay procedure (4). Sterile deionized water (100 ml) adjusted to contain 10^8 PFU/ml was passed through each resin column in 15 min. Plaque counts of the effluent were carried out as described above. Triplicate TSA plates were prepared for each dilution.

Bacterial survival and filtration capabilities in the SDB resin. Fourteen duplicate columns were prepared and each pair was numbered 1 to 14. Twelve pairs contained the washed, autoclaved, air-dried, sodium-regenerated resin (SDB); two pairs contained the calcium-regenerated resin. All resin columns were incubated at room temperature. The resin beds were prepared and tested for sterility as described above. Two suspensions of *Pseudomonas fluorescens*, each washed three times and adjusted to contain 10^8 CFU/ml in 100 ml deionized water, were passed through columns no. 1. Triplicate plate counts (TSA) as described above were made from the influent and effluent waters. Tap water (250 ml) was passed through all columns no. through 17. This was repeated every other day for 76 days. On the 6th day following initial passage of *Pseudomonas*, triplicate plates were prepared after 100 ml of sterile, deionized water was passed through each of the no. 1 columns. On the 13th day, two 100-ml samples of freshly cultured and washed (10^8 CFU/ml) *P. fluorescens* suspension were passed through each of the no. 2 columns. Triplicate plate counts of effluent dilutions were again prepared. On the 22nd and 37th days, the same procedure was repeated for columns no. 3 and 4, respectively. Furthermore, on the days mentioned, triplicate plates were also prepared from effluents of columns previously seeded. The procedures, as described above, were repeated for columns no. 5 through 8 using *Staphylococcus aureus*. Columns no. 9 and 10 contained the SDB calcium-regenerated resins seeded with *Pseudomonas* and *Staphylococcus*, respectively, on the 1st day only. A half-strength solution of nutrient broth was passed through columns no. 11

and 12 on the 1st day. These columns were similarly seeded with *P. fluorescens* and *S. aureus*, respectively. Columns no. 13 and 14 served as control columns; tap water only (no. 13) and sterile tap water only (no. 14) was passed through every other day. Triplicate plate counts were prepared from the effluent dilutions of columns no. 9–14 at the same intervals mentioned above, and on all columns on days 37, 51, 64, and 77. Finally, on days 80 and 81, all resin beds were cultured for microorganisms at depths of 1, 8, and 16 cm. A piece of sterile glass tubing (diam 8 mm) was inserted into the resin column and a measured amount was dispensed into a sterile 9-ml water blank. The tubes were shaken vigorously for 30 sec to elute the organisms. Serial 10-fold dilutions were prepared from the supernatant fluid; triplicate plate counts were then made for each dilution to determine the number of colonies/g of resin. Similar measured samples of resin were dried and weighed.

Influent and effluent water analysis. On a weekly basis, measurements of the *p*H, chlorine, and total hardness of both influent and effluent waters were determined for each column. Measurements were made using kits provided by the Hach Chemical Co. Furthermore, the amount of protein in both influent and effluent waters was determined using the modified technique of Lowry et al. (18), with bovine-albumin fraction V as the standard.

RESULTS AND DISCUSSION

Microbial content of water-softener units. The backflushed samples from 143 separate exhausted water softener tank resins were examined, exclusive of the control tanks and salt brine samples.

The total numbers of microorganisms varied from 0 to 3 × 10^4/100 ml. Most samples contained 10 to 100 CFU/100 ml. No microorganisms were isolated from 17 exhausted tank resins (15 urban, 1 rural, 1 dairy) and eight of the 12 control tanks. All units servicing rural homes contained over 1,000 CFU/100 ml, with eight having 30,000 CFU/100 ml.

Forty-four different genera of bacteria and fungi were isolated from all samples tested. The tank source and 27 of the different microorganisms are summarized in Table 1. *Bacillus*, *Candida*, *Clostridium*, and *Staphylococcus* appear most often (Group 1). Additional genera are listed in order of decreasing frequency included *Aerobacter*, *Alcaligenes*, *Aspergillus*, *Brevibacterium*, *Cryptococcus*, *Geotrichum*, *Micrococcus*, *Paecilomyces*, *Paracolobactrum*, *Penicillium*, *Rhodotorula*, *Serratia*, and *Streptomyces*. Seventeen genera representative of tanks from which only three or fewer isolates were cultured were not included.

Two different staphylococci, a gram-negative diplococcus, a gram-negative coccus, pleomorphic gram-negative rods, *Bacillus* and a *Corynebacterium* were repeatedly cultured from the stock salt brine. On two occasions we cultured a *Thermoactinomyces*. The most prevalent microorganisms were gram-negative diplococci which became pleomorphic upon subculture. The diplococci failed to grow at 37 C, grew slowly at 4 C, and grew optimally at room temperature. A *Staphylococcus*, *Bacillus*, and a *Flavobacterium* sp. were isolated from four of the 12 samples of the cleaned (backflushed, sanitized, regenerated, and washed) control tank resins. Identification of urban tank microorganisms revealed several interesting phenomena. During a 6 to 7 month period, the same tanks are backflushed and cleaned 4 to 5 times. Microbial assessments were completed (four samples) on the same tank servicing different units. *Mycobacterium* and a *Pseudomonas* sp. were isolated the four times the tank was returned for cleaning. *Alcaligenes* and *Brevibacterium* sp. were also repeatedly isolated on three occasions from another tank. Furthermore, similar microorganisms were cultured from different tanks servicing the same dwelling, indicating a persistent source of resin contamination from this unit. Two such studies with five tanks revealed the presence of *Aspergillus* sp. and *Paecilomyces* sp. Furthermore, a home was contaminated by a fourth tank after three previous tanks servicing the same unit indicated an absence of microorganisms.

Unfortunately, for comparative purposes, similar field studies have not been published. Nevertheless, these studies indicate that the SDB resins remove and support the growth of bacterial and fungal microorganisms, that exhausted tank resins in particular are heavily contaminated, that microorganisms are indigenous and are not all destroyed in these 9-inch tanks of resins. It is of paramount importance, therefore, that the sanitization procedure using calcium hypochlorite (1.7 gm/ft³ of resin) or chloromelamine (4.5 gm/ft³ of resin), during the salt regeneration process destroys these organisms. Moreover, as a requisite for an effective cleaning procedure, the backwash component, a representative number of the cleaned tanks should periodically be cultured. This procedure would also permit the use of that particular tank resin by more definitively monitoring the microflora of the plumbing and water supply of that premise.

Although it is true that the public health menace posed by microbial growths in ion exchange resins is very slight, certain microorganisms which we have uncovered are noteworthy (*see* Introduction).

The more definitive study characterizing the

TABLE 1. *Bacteria and fungi isolated from the backflush contents of 143 water softening units, 12 samples of stock salt brine, and 12 control tank resins*

Microorganism	Total no. of isolates	No. of isolates from					
		Farm	Private home	Hospital	Dairy	Stock salt brine	Control tank resins
Achromobacter	12	1	7	4			
Aerobacter	5	1	4				
Alcaligenes	8	1	6	1			
Aspergillus	8	1	7				
Bacillus	37	5	27	2	3	12	4
Brevibacterium	6	1	4	1			
Candida	16		16				
Clostridium	25	7	18				
Corynebacterium	12		12			10	
Cryptococcus	5	3	2				
Escherichia	12	3	9				
Flavobacterium	13	1	6	6			3
Geotrichum	6	6					
Micrococcus	8		8				
Mycobacterium	10		8	1	1		
Paecilomyces	8	2	6				
Paracolobactrum	8	1	5	2			
Penicillium	6		6				
Pseudomonas	9		7	1	1		
Rhodotorula	8	1	7				
Sarcina	11	1	10				
Serratia	6		6				
Staphylococcus	33	8	25			10	4
Streptococcus	11	1	10				
Streptomyces	5		5				
Gram-negative coccus	12					12	
Gram-negative diplococcus	12					12	

staphylococcal salt brine isolate was also interesting. This organism appeared as a medium-size, white, glistening, convex, entirely edged colony on BHI agar. The optimal growth temperature was 37 C. Acid was produced from glucose, lactose, and sucrose; mannitol was not fermented. Growth was not observed on MS agar or nutrient agar. The catalase and MR tests were positive, acetoin was produced from glucose, nitrates were reduced, and litmus milk was acidified and coagulated. Furthermore, phenylphosphate was hydrolyzed, and deoxyribonuclease and fibrinolysin activity was demonstrated. Beta-type lysis occurred on rabbit and human BA, but not on sheep BA. Sufficient free and bound coagulase was also demonstrated. Plaque development was absent for all 24 phages. The tube hemolysin titrations indicated guinea pig RBC to be the most susceptible; the next most susceptible were those of the rabbit and human, and then the moderately susceptible sheep cells. Ox cells were not lysed. These titrations indicated a delta-type staphylococcal hemolysin. The isolate was resistant to tetracycline, neomycin, triple sulfa, sulfamethoxypyridazine, and sulfamethizole.

Within 24 to 48 hr the ID injections produced inflammatory areas approximately 4.5 cm in diameter. This area receded within 6 days and produced an area of necrosis. All signs of inflammation were absent 8 days after injection. All rabbits survived the intravenous injections with no apparent sign of illness. All mice died within 6 to 30 hr after intraperitoneal injections. The peritoneal cavities were covered with a white mucous substance and staphylococci were isolated from both peritoneal fluid and heart blood.

Since the *Staphylococcus* was not susceptible to the phages, the most definitive test to relate cleaned and exhausted softener resin and salt brine staphylococcal isolates could not be used.

In spite of the absence of an alpha hemolysin and pigmentation, and in spite of the failure to ferment mannitol aerobically and to liquefy gelatin, the above cultural characteristics are suggestive of the pathogenic staphylococci. For

example, strains are generally considered pathogenic if they produce both free and bound coagulase (11). Correlation has also been reported between phosphatase synthesis and pathogenicity (2), and between deoxyribonuclease and coagulase activity (25).

Cation effect on bacterial filtration. All ion exchanged resin columns removed bacteria (Table 2). Comparable percentages of both the gram-positive *Staphylococcus* and the gram-negative *Flavobacterium* were filtered from the suspension. In addition to the massive bacterial destruction by the Ag^{+}-regenerated resin, Pb^{2+} removed over one half of the total numbers of organisms. The Na^{+}, along with Fe^{2+}- and Al^{3+}-regenerated resins, removed approximately a third of the organisms. The Cu^{2+} and Ca^{2+} resins filtered a much smaller percentage of each suspension.

Cations, upon accumulation in the resin bed, can change the filtering characteristics. A smaller microbial filtration capability would occur for a Ca^{2+}- or Cu^{2+}-spent resin than for a Na^{+}-, Fe^{2+}-, Pb^{2+}-, or Al^{3+}-exhausted resin.

Except for the Ag^{+}- regenerated resin, these same microorganisms were cultured from all resin effluent waters after 3 days. Dissolution of this metallic ion in water to the extent of 10^{-5} g/liter is bactericidal. Bacterial counts decreased exponentially in all columns thereafter, and, on day 13, all resin effluents, except Ca^{++}, showed an absence of microorganisms. This is noteworthy because the calcium ion protects microorganisms against disinfecting agents (20). Furthermore, as the resin becomes exhausted (i.e., accumulates calcium), the maintenance of entrapped microorganisms is prolonged. Nevertheless, these results confirm the observations of Klumb (17), namely, that siliceous resins in the absence of accumulated, filtered organic matter are incapable of sustaining bacterial growth.

Our experimental design with viable organisms failed to reveal any significant role of valency in bacterial resin adsorption. It has been shown (14) that staphylococcal cell wall suspensions bind metal ions and that divalent ions showed a greater affinity for cell walls than monovalent ions. Furthermore, affinity increased with atomic weight. Others (27) observed that positively charged surfaces are more conducive to the attachment of a gram-negative bacteria such as *Escherichia coli*, than negatively charged ones. Puck and Sagik (22) confirmed the existence of negatively charged binding groups on the surface of *Escherichia coli* B cells at *p*H 7. These cells became rapidly attached to a positively charged sulfonic-acid polystyrene resin, but not to a negatively charged one. Obviously, bacterial surface charges alone do not govern adsorption (24). Cell configuration, relative position of the charges, age of the cells, and type of resin used are equally important. Moreover, cell wall constituents, such as diaminopimelic acid, various amino acids, teichoic acid, or hexosamine, may provide the necessary charges for adsorption to the resin surface.

Cation effect on bacteriophage filtration. The Mn^{2+}- and Pb^{2+}-regenerated resins failed to adsorb the phage (Table 3). Moderate differences in the affluent and effluent plaque counts were observed with the Na^{+}- and Ca^{2+}-regenerated resins. Significant decreases, however, occurred with Fe^{2+}, Cu^{2+}, Al^{3+}, and the obvious destruction of bacteriophage with Ag^{+}.

In a somewhat similar study (22), using a cationic resin of the sulfonic-acid type regenerated with 10% NaCl, it was demonstrated that at least an 0.15 M NaCl concentration was required for adsorption of T1 and T2 *Escherichia coli*

TABLE 2. *Capacity of the SDB resin column (Na^{+}) and SDB resins, exchanged with mono-, di-, and trivalent cations, to remove microorganisms from a suspension containing 10^{6} CFU/ml*[a]

Atomic weights	Resin cations	Per cent of organisms removed	
		Staphylococcus	*Flavobacterium*
22.9	Na^{+}	36	33
107.8	Ag^{+}	99	99
55.85	Fe^{++}	35	31
207.21	Pb^{++}	56	60
63.57	Cu^{++}	10	15
40.08	Ca^{++}	14	12
26.97	Al^{+++}	34	35

[a] Results are averages of triplicate-column runs.

TABLE 3. *Capacity of the SDB resin column (Na^{+}) and SDB resins, exchanged with mono-, di-, and trivalent cations, to remove Escherichia coli B bacteriophage from a suspension containing 10^{6} PFU/ml*[a]

Resin cations	Per cent of bacteriophage removed
Na^{+}	31
Fe^{++}	70
Ag^{+}	99
Cu^{++}	74
Ca^{++}	25
Al^{+++}	84

[a] Results are averages of triplicate column runs.

bacteriophage and influenza virus. Furthermore, after attachment, splitting of the T2 phage into its protein and DNA components occurred.

Our primary interest was resin filtration of bacteriophage. Future studies will determine whether the decreases observed were due to actual adsorption, inactivation, destruction, or phage splitting by the various exchanged resins.

Bacterial filtration and survival capabilities in resin. After testing innumerable tap water outlets in laboratories, we were unable to find one free of a *Flavobacterium*. The tap water concentration of this organism was approximately 200 CFU/100 ml. The faucet outlet was swabbed with alcohol and the water permitted to run for 5 to 10 min before the 250-ml sample was taken and passed through the columns.

Initial column filtration results are shown in Fig. 1. Effluent counts are given in Fig. 2 for *Pseudomonas* and in Fig. 3 for *Staphylococcus*.

The removal of bacterial suspensions by the SDB resin differed significantly according to the amount of tap water passed through each column (Fig. 1). The initial filtration percentages (day 0)

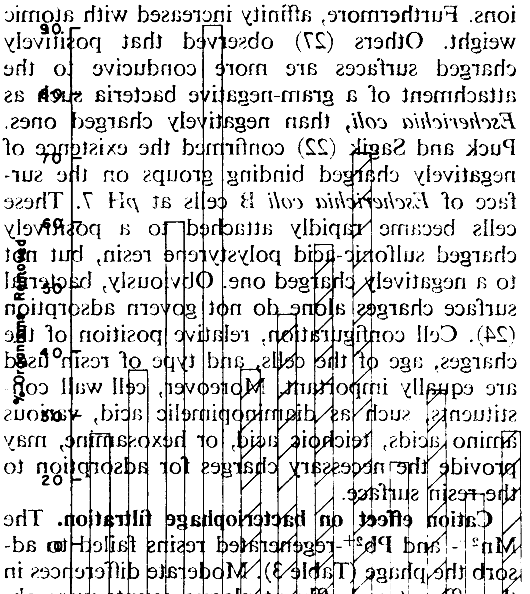

FIG. 1. *Removal of Pseudomonas fluorescens, paired columns 1–4 (open bar), and Staphylococcus aureus, paired columns 5–8 (crossed-bar) with the sodium cationic SDB resin. Paired columns 9, 10 are the SDB calcium-exchanged resins. Paired columns 11, 12 represent the sodium SDB resin treated with one half strength nutrient broth.*

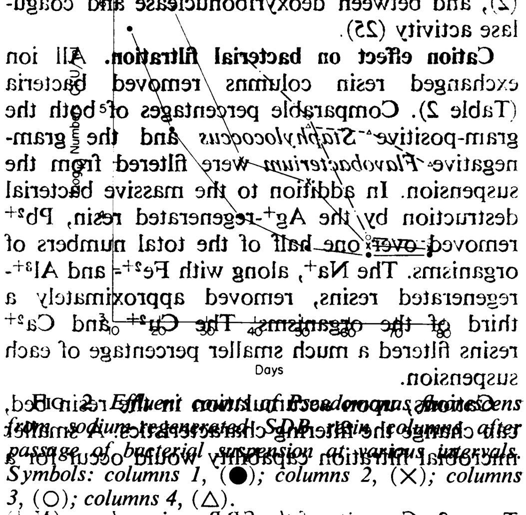

FIG. 2. *Effluent counts of Pseudomonas fluorescens from sodium-regenerated SDB resin columns after passage of bacterial suspension at various intervals. Symbols: columns 1, (●); columns 2, (×); columns 3, (○); columns 4, (△).*

shown in column 1 (*Pseudomonas*) and column 5 (*Staphylococcus*) were favorable compared with values obtained with the Na^+ resin in the previous study (Table 2) with sterile deionized water. Values were 28% for *Pseudomonas*, 33% for *Flavobacterium*, and 37% and 36% for the *Staphylococcus*. Slightly less bacterial retention was observed with the calcium-regenerated resin (columns 9, 10), i.e., 22% and 33% of the *Pseudomonas* and *Staphylococcus*, respectively. Only 18% and 28%, respectively, were removed by the nutrient-broth treated resins (columns 11, 12). The initial filtration studies (columns 1, 5, 9, 10, 11, 12) indicated the capacity of the resin to remove more of the gram-positive than the gram-negative organisms. However, when compared with *Pseudomonas*, less retention of the gram-positive organisms occurred within the resin over the 37-day period. In every case, as the volume of tap water passed through each column every other day increased, the numbers of bacteria removed by the resin also increased. After 37 days, for example, column 4 retained 91% of the *Pseudomonas* suspension, whereas approximately 72% of the *Staphylococcus* was removed. Undoubtedly, the accumulation of organic materials and inorganic materials, such as the exchanged magnesium, calcium, and other cations which accumulate in the resin, contributed to this retention.

Both microorganisms survived and were released in fairly large numbers into the effluent waters throughout the entire 77-day study (Fig.

2, 3). The effluent counts of *Pseudomonas* (columns 1 to 4) showed a gradual decrease. The initial count of $\times 10^6$ CFU/ml slowly decreased and maintained a plateau after about 64 days of slightly less than 10^4 CFU/ml. Column 4 effluent counts taken during the last 22-day period showed a moderately larger count. Although the initial staphylococcal counts were slightly lower, the

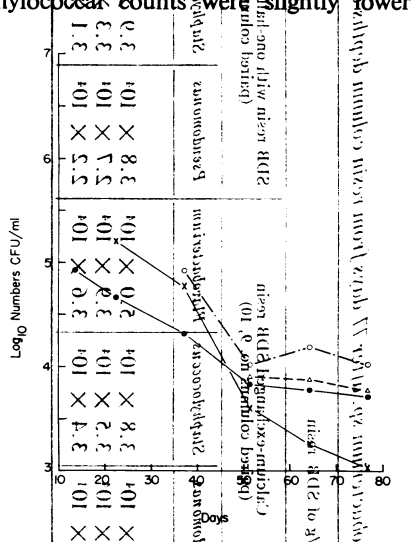

FIG. 3. *Effluent counts of Staphylococcus aureus from sodium-regenerated SDB columns after passage of bacterial suspension at various times. Symbols: columns 5, (●); columns 6, (×); columns 7, (○); columns 8, (△).*

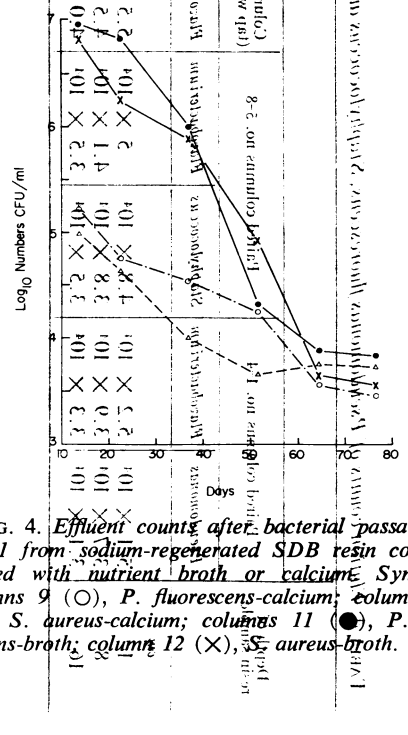

FIG. 4. *Effluent counts after bacterial passage on day 1 from sodium-regenerated SDB resin columns treated with nutrient broth or calcium. Symbols: columns 9 (○), P. fluorescens-calcium; columns 10 (△), S. aureus-calcium; columns 11 (●), P. fluorescens-broth; column 12 (×), S. aureus-broth.*

sults described above with the 13-day resin columns and sterile deionized water.

Effluent counts of *Pseudomonas* and *Staphylococcus* from columns treated with calcium (columns 9, 10) and nutrient broth (columns 11, 12) also declined and appeared to level off at approximately 10^4 CFU/ml after 64 days (Fig. 4). A 1-log increase for both microorganisms occurred in the nutrient broth columns, i.e., from an initial inoculum of 10^6 to approximately 10^7 on day 13. However, both declined to population levels comparable to other columns. The influence of the nutrient broth and the calcium did not enhance the survival characteristics of these two bacteria.

Effluent *Flavobacterium* counts increased with the amount of tap water passage and leveled off at approximately 10^5 CFU/ml of effluent (Fig. 5, 6). This undoubtedly reflects the maximal population that our resin column will support. *Flavobacterium* counts from the effluents of *Pseudomonas* seeded columns 1–4 and tap water control columns 13 and the staphylococcal percolate, columns 5–8, are shown in Fig. 5 and 6, respectively.

Flavobacterium counts from columns 1 and 2, seeded with *Pseudomonas* on day 0 and 13, respectively, showed a steady 6-log increase, achieving the maximal stationary level of approximately 10^6 CFU/ml on days 35 and 50, respectively (Fig. 5). *Pseudomonas* (columns 3 and 4), seeded on days 22 and 37, showed lower *Flavobacterium* counts (approximately 4.5-log increase) at days 37 and 50, respectively. Comparable numbers were maintained over the remaining time period of 30 days. The unseeded columns (no. 13, tap water passage only) showed a slow 4-log increase achieving a concentration of approximately $10^{4.5}$ CFU/ml after 60 days, with the major increase occurring over a 37-day period. The final cell concentration was comparable to that achieved in columns 3 and 4.

Several factors contribute to the enhanced *Flavobacterium* population in columns 1 and 2. For example, more *Pseudomonas* microorganisms were retained within the resin when columns 3 and 4 were seeded on days 22 and 37 (Fig. 1). Competition for available substrates could cause the reduced *Flavobacterium* population. Furthermore, we have established the direct relationship between the accumulated, tap-water exchanged calcium, magnesium, and other cations in the resin and the increased microbial retention (Fig. 1).

The staphylococcal columns (no. 5 and 6) seeded on day 0 and 13, respectively, did not show the same phenomenon (Fig. 6). The *Flavobacterium* in column 5 showed an early 3.5-log increase after 13 days and a 4.6-log increase at 22 days, before maintaining a relatively

TABLE 4. *Elution counts of Pseudomonas fluorescens, Staphylococcus aureus and Flavobacterium sp. after 77 days from resin column depths of 1, 8, and 16 cm*

Depth of resin sample	Average CFU/g of SDB resin											
	Paired columns no. 1-4		Paired columns no. 5-8		Column no. 13 (tap water only)	Calcium-exchanged SDB resin (paired columns no. 9, 10)			SDB resin with one-half strength nutrient broth (paired columns no. 11, 12)			
	Pseudomonas	*Flavobacterium*	*Staphylococcus*	*Flavobacterium*	*Flavobacterium*	*Pseudomonas*	*Staphylococcus*	*Flavobacterium*	*Pseudomonas*	*Staphylococcus*	*Flavobacterium*	
cm												
1	5.1×10^4	5.2×10^4	4.8×10^4	5×10^4	5.5×10^4	4.1×10^4	3.8×10^4	5.0×10^4	3.8×10^4	3.9×10^4	6.1×10^4	
8	3.7×10^4	3.9×10^4	3.8×10^4	4.1×10^4	4.5×10^4	3.6×10^4	3.5×10^4	3.9×10^4	2.7×10^4	3.3×10^4	4.9×10^4	
16	3.1×10^4	3.3×10^4	3.5×10^4	3.5×10^4	4.0×10^4	3.5×10^4	3.4×10^4	3.6×10^4	2.2×10^4	3.1×10^4	4.8×10^4	

consistent population for the additional 55 days. Bacterial multiplication in column 6 was slower. A 3.5-log increase occurred after 22 days, with an additional gradual increase for approximately 40 days before acheiving 50,000 CFU/ml. The larger numbers of *Staphylococcus* adsorbed by the resin seeded on day 13 contributed to this slower increase. However, column 7, *Flavobacterium* numbers (37 days) showed similarities with column 5. Column 8 counts were highest on day 51. A 5.5-log increase was observed which decreased slightly over the 27-day period thereafter. The fact that columns 7 contained more *Staphylococcus* after day 22 than columns 5 did not have the same depletion effect on the indigenous *Flavobacterium*. The cocci added to column 8 on day 37 caused a slight stimulatory effect. Columns 14, through which sterile tap water passed for 77 days, showed no counts at any time.

Obviously, effluent counts alone are inadequate to disclose the extent of microbial growth. The microorganisms are not readily dislodged because they are adsorbed to the resin and enmeshed within the resin matrix.

After 80 days, it was evident that considerable numbers of *Pseudomonas*, *Staphylococcus*, and *Flavobacterium* were not washed from the resin with the passage of 250 ml of tap water (Table 4). The average *Pseudomonas* counts from the top 1-cm layer of the four paired resin columns (1–4) and the staphylococcal paired resin columns (5–8) contained 5.1×10^4 and 4.8×10^4 CFU/g of resin, respectively. At depths of 8 cm, the average counts of these same bacteria were 3.7×10^4 and 3.8×10^4 CFU/g of resin; at 16 cm, the average counts were 3.1×10^4 and 3.5×10^4 CFU/g of resin.

Bacterial diminution with increased resin depths was also observed with the *Flavobacterium* (Table 4) cultured from both the *Pseudomonas*- and *Staphylococcus*-seeded columns 1–4 and 5–8, respectively. A similar reduction was apparent with columns 13, through which tap water passed only, although slightly larger numbers of *Flavobacterium* were enmeshed within the resin.

Bacterial multiplication within these small resin columns is best illustrated with paired columns 1, 5, and 13; i.e., resin columns which had been exposed to *Pseudomonas* and *Staphylococcus*, respectively, prior to the addition of tap water (Fig. 1) and miroorganism indigenous to tap water. Elution procedures completed on day 81 on the entire 20 g in each paired resin column revealed average counts of 4×10^4 CFU/g of resin, or 8×10^5 organisms/column no. 1. Since only 28% of the 10^6 bacterial suspension was removed after the only initial bacterial passage on day 0, the above results represent more than a threefold increase, i.e., from 2.8×10^5 CFU/ml (day 0) to 8×10^5 of the total number of cells (day 81). A smaller twofold increase was observed with the *Staphylococcus*, i.e., 3.8×10^5 to 8.5×10^5 total numbers of cells. Furthermore, effluent counts taken on the same day would increase the total number of cells in both instances 1.5×10^5 (10^4 CFU/ml $\times$ 15 ml).

The total inoculum for the indigenous tap water organism over the 80-day period (500 CFU/250 ml every other day) was 1.9×10^4 cells. A moderate increase was shown with the effluent count of 4.8×10^4 CFU/ml. Elution tests, however, showed 5.1×10^4 CFU/g of resin, or slightly over 10^6 colonies/resin column. Under our experimental conditions it is apparent that at room temperature this resin can support the simultaneous growth and multiplication of several different types of microorganisms.

When compared to columns 1–4 (Table 4) fewer *Pseudomonas* were eluted from the 1, 8, and 16 cm depths of the SDB resin column exposed to nutrient broth. The same reduced numbers were apparent with the *Staphylococcus*. However, the *Flavobacterium* counts were slightly higher than counts observed for column 13. With the exception of the resin, eluate counts taken at 1-cm depths, the *Pseudomonas* and *Staphylococcus* counts from the calcium-regenerated resin were comparable to counts obtained from paired columns 1–4 and 5–8. Furthermore, calcium either had a slight inhibitory effect on the growth of the *Flavobacterium* or reduced its adsorptive abilities, since the lowest numbers of CFU/g of resin were detected in this resin.

No change occurred in the *p*H of the influent and effluent waters. Consistent *p*H values of 6.8 to 7.1 were obtained. Influent tap water contained 1 ppm of chlorine. No chlorine was detected in samples of the effluent water, indicating removal by the filtered organic matter and the SDB resin. Chlorine in the resin matrix had no marked bactericidal effect upon the seeded microorganisms or the *Flavobacterium*.

Total hardness values indicated that the resin continued to efficiently soften water throughout the study period. Protein levels of column effluent waters, over the 77 day period, increased from 3 μg to approximately 25 μg of protein/ml.

Adsorbed or filtered organic matter would be more abundant in the upper layers of the resin, resulting in the establishment of a larger microbial population. Cation exchangers of the sulfonic type have been shown to take up all cations, both organic and inorganic (8). Ampholytic chemicals, such as amino acids, are also removed (24). Furthermore, it is reasonable to conclude that solid surfaces, such as resin particles, enable bacteria to develop in substrates otherwise too dilute for growth, by adsorbing and concentrating organic matter (15). Once bacterial growth is

established on such surfaces, the rate of biological reactions is accelerated. In addition to concentrating nutrients by adsorption, and providing a resting place for bacteria, solid surfaces also retard the diffusion of exoenzymes and hydrolysates away from the cell, thereby promoting assimilation (27).

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